

FH HES

Universities of Applied Sciences

Fachhochschulen – Hautes Ecoles Spécialisées

Continuous Processes and Flow Chemistry at the Universities of Applied Sciences in Switzerland

Christophe Allemann^a, Roger Marti^a, Olivier Vorlet^a, Oliver Martin^b, Peter Riedlberger^b, Tobias Leonhardt^c, Angelo Gössi^c, Wolfgang Riedl^c, Jean-Manuel Segura^d, Manfred Zinn^d, and Simon Crelier^d

^aCorrespondence: Prof. Dr. C. Allemann^a, E-mail: Christophe.allemann@hefr.ch; ^aHES-SO Fribourg, University of Applied Sciences Western Switzerland, Haute Ecole d'Ingénierie et d'Architecture Fribourg, Institute of Chemical Technology, Boulevard de Pérolles 80, CH-1705 Fribourg; ^bZurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Research Group Chemical Engineering, Einsiedlerstrasse 31, CH-8820 Wädenswil; ^cHochschule für Life Sciences, Institut für Chemie und Bioanalytik, Gründenstrasse 40, CH-4132 Muttenz; ^dHES-SO Valais Wallis, University of Applied Sciences and Arts Western Switzerland, Institute of Life Technologies, Route du Rawyl 47, CH-1950 Sion

Abstract: This article provides an overview of activities in the fields of continuous processes, flow chemistry and microreactors at the Universities of Applied Sciences in Switzerland.

Keywords: Applied Research and Development · Downstream Processing · Flow Chemistry · Microreactors · PAT

1. Introduction

Continuous (bio)chemical processes, applying continuously flowing streams, have been known for a long time and are commonly used to prepare chemical commodities at large scale. However, most operations in pharmaceutical and fine chemical industries are still routinely performed batchwise. Nevertheless, continuous processes offer great benefits not only at laboratory but also at production scale: they are cost effective,^[1,2] safe,^[3,4] reliable, and they allow reduced time-to-market,^[5] high productivity, flexibility, efficient scale-up,^[6] as well as minimized ecological impact.^[7] Hence, over the last decade both industry and academia have recognized the huge potential to produce pharmaceuticals and fine chemicals in continuous reactors.

In view of this enormous potential impact on the (bio)chemical industry, the Universities of Applied Sciences in Switzerland are developing new continuous reactions, novel downstream processes (DSP), and innovative process analytical technologies (PAT). Besides continuously stirred tank reactors (CSTR) and plug flow reactors (PFR), the emergence of process intensification delivered new methods and equipment to perform (bio)chemical reactions (e.g. centrifugal contactor separators (CCS), capillary reactors, microreactors).^[7,8] With these new tools, various heterogeneous and homogeneous reactions catalyzed as well as uncatalyzed are investigated. Moreover, strategies to deal with solids formation in microchannels are devised and continuous DSP for precipitation, crystallization, liquid–liquid extraction, and membrane separation are developed. Furthermore, studies on the micromixing impact on mass transfer or product quality and the development of new online analysis approaches (including bio-analytics) to improve the control of flow processes are undertaken. A few examples of these activities are described in this paper.

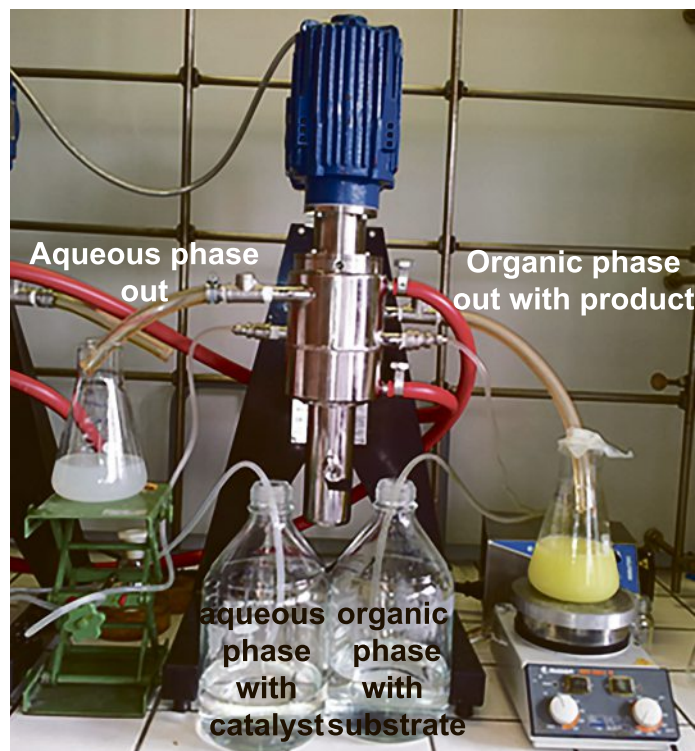


Fig. 1. CCS used for biphasic aldol reactions.

2. HES-SO – Haute Ecole d'Ingénierie et d'Architecture de Fribourg

At the Haute Ecole d'Ingénierie et d'Architecture de Fribourg,^[9] the importance of continuous processes is emphasized in education as well as in research. Bachelor and Master students in chemistry are intensively trained in flow chemistry. Moreover, two of the three main research focus of the Institute of Chemical Technology (ChemTech)^[10] are related to the area of continuous processes and flow chemistry. Continuous operations at laboratory and pilot plant scale are routinely performed. There are many successful examples in our laboratories on the transfer of reactions from batch to continuous mode. In all cases, careful evaluation of the reaction conditions (like solid formation, reaction kinetics, and thermal behavior) sets the basis for the design of an appropriate microreactor set-up. Optimization of reaction temperature and residence time as well as productivity lead to a flow process; examples are high-temperature Diels–Alder reactions or organocatalytic oxa-Diels–Alder reactions, Kumada reactions, Wittig reactions, polymerization reactions, reduction and oxidation reactions, phase-transfer-catalyzed alkylations,^[11] or the very challenging gas–liquid photooxidation at low O₂ pressure (<3 bars)^[12] of α -pinene into pinovarvone, a model reaction to develop our photochemical capabilities in coiled and chip reactors.

Continuous processes are not limited to microfluidic devices, and larger setups like centrifugal contactor separator (CSS) are used in ChemTech (Fig. 1). A CCS is a modified centrifuge that works as a multifunctional reactor to perform biphasic reactions

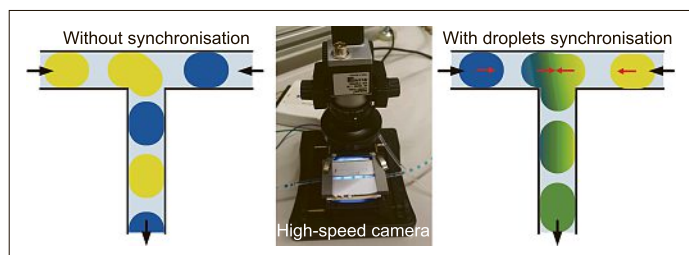


Fig 2. When droplets are not synchronized, the reaction does not proceed due to poor mixing (left). A high-speed camera (center) is used online to synchronize segmented flow so that the reaction occurs (right).

and separation in the same unit.^[13] It has been successfully used to perform several reactions like the alkylation of *p*-methoxyphenol with allylbromides or the aldol reaction between methyl isobutyl ketone and benzaldehyde.^[14] Optimization of these reactions is ongoing.

ChemTech is also developing technologies like PAT to understand and control the flow pattern in order to improve mixing processes. The segmented flow regime is particularly interesting for the production of well-defined nanoparticles. A high-speed camera is used as an online tool to determine the exact shape, volume, position, and velocity of each droplet before the mixing point (Fig. 2). This information is used to control the flowrate of each pump in order to ensure synchronized droplet coalescence for perfect mixing.

3. ZHAW – Zurich University of Applied Sciences

Optimizing microreactor technologies for the utilization of biomass exhibits great potential towards a ‘greener’ chemical industry.^[15] Numerous catalyzed reactions in such systems have been reported. However, their application in the preparation of heterogeneous catalysts required for upgrading biomass has been greatly overlooked, although the controlled synthesis of catalysts is key for improving efficiency and sustainability of the processes which they are used in.^[16] One crucial aspect impeding the production of solids in microreactors is the occurrence of fouling, *i.e.*, clogging of the μm – mm -sized reactor channels. Therefore, the Research Group Chemical Engineering at the ZHAW focuses on developing microreactor based processes for *i)* the synthesis of solid catalysts and, additionally, *ii)* their subsequent use in biomass conversion (Fig. 3).

Continuous synthesis of catalysts enables the facile addition of modifying agents at any stage as well as precise adjustment

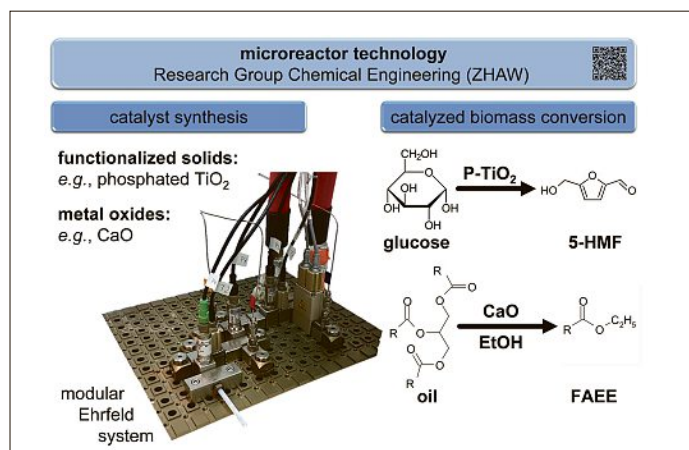


Fig. 3. Fields of investigation by the Research Group Chemical Engineering at the ZHAW in Wädenswil. The image shows one of our microreactor setups for the synthesis of phosphate modified TiO_2 via sol-gel. FAEE: fatty acid ethyl ester.

of process parameters to tune the resulting catalyst properties (*e.g.* basicity/acidity, surface area, particle size, *etc.*), which ensures a stable product quality. Besides product control, biomass conversion in microreactors can be operated much more safely compared to batch mode and supersedes the time and cost intensive scale-up of the catalyst and process. In addition, design-of-experiments (DoE) is applied to establish relationships between synthesis, catalyst features, and catalytic performance with a minimum of experiments to be conducted for optimizing catalyst production, catalyst properties, and catalyzed reaction. This provides a better understanding of the chemical reactions and hence, further improvement of the microreactor processes (*e.g.* to prevent fouling or deactivation of the catalyst). Based on this approach, optimal catalyst preparation and optimal heterogeneously catalyzed biomass conversion processes could be developed such as the synthesis of the platform chemical 5-hydroxymethylfurfural (5-HMF) from glucose over phosphated TiO_2 or the production of biodiesel from microalgal fats over CaO .

4. FHNW – School of Life Sciences

Batch versus continuous operation is a controversial issue in the life science industry. Recent developments in simulation tools, material science, and production technologies, especially in the field of additive manufacturing, resume the discussion. At the School of Life Sciences FHNW (HLS) in Muttenz, a project funded by F. Hoffmann-La Roche AG deals with the use of novel 3D plotted plug-flow reactors for process intensification. Such reactors allow an excellent heat transfer, good mixing characteristics, and a narrow residence time distribution.^[17] Thus, a catalyzed oxidative cyclization^[18] was chosen as a suitable model reaction to be carried out with this pilot plant unit (Fig. 4). Conventionally, this reaction is carried out in batch mode with a homogeneous catalyst and with air instead of oxygen enriched gas phase, for safety reasons. With the use of the easy scalable 3D plotted plug-flow reactors however, the catalyst can be immobilized at the reactor surface directly. This, in combination with the very good heat transfer, allows the reaction to be carried out with pure oxygen. Evaluations show an expected reaction time of 10–15 minutes with the new reactor setup instead of 20–24 hours using conventional batch stirred tank reactors. With a 7 mm diameter 3D-plotted tubular reactor up to 15 kg/h of product can be generated, similar to the product quantity which can be generated in a 3 m³ stirred tank per hour.^[19]

In many cases, dedicated downstream processing (DSP) needs to be implemented after the (continuous) reaction in order to purify the product by the removal of both undesired by-products and solvent. At the HLS the use of membrane technologies for

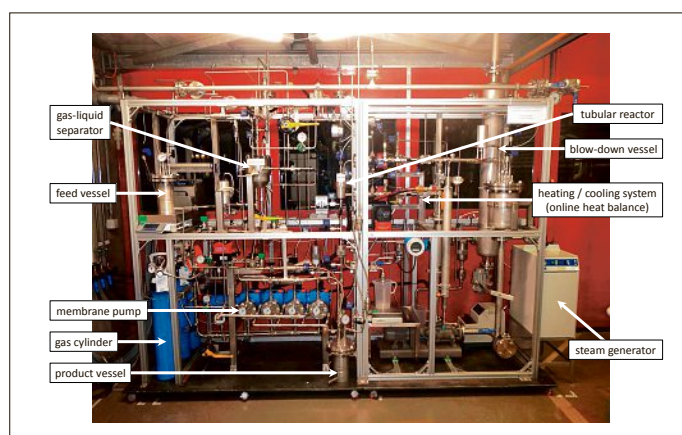


Fig. 4. Novel reactor set-up with 3D plotted tubular reactor.

DSP purposes is extensively investigated, besides others.^[20] In combination with inline measurement (*e.g.* refractometric index, density, conductivity) a process chain from the product generation in (innovative) continuous reactors followed by continuous DSP-technologies can be assembled and is in use for industrial projects as well as for educational purposes.

5. HES-SO Valais-Wallis – Institute of Life Technologies

This section highlights some of the applications that were investigated at the Institute of Life Technologies of the HES-SO Valais-Wallis.

In *Bioanalytics*, flow injection analysis using microfluidics is an attractive approach enabling automatized and rapid analytical preparation using minute amounts of sample. This approach was applied to the rapid mixing of proteins with denaturants in a microfluidic circuit to explore protein folding using Fourier-Transform Infrared Spectroscopy (FTIR).^[21] Another development tackled the (quasi) real-time monitoring of fermentation processes (PAT), which is highly demanded in the biopharmaceutical industry. In this respect, a successful collaboration between HES-SO Valais Wallis and HES-SO Fribourg^[10] led to the development of a quick and specific analytical method to follow the production of His-tagged proteins.^[22] The new method is based on the binding of a specific fluorescent probe to the recombinant proteins released after cell lysis, with a read-out based on fluorescence polarization. Proof of principle was obtained that this assay can be integrated in a lab-on-valve (LOV) setup with automated lysis and mixing, followed by detection in a microfluidic platform (Fig. 5).

Monitoring of cell growth and PHA production using flow cytometry. Many wild-type bacteria are able to accumulate the intracellular carbon and energy storage compound poly(3-hydroxyalkanoate) (PHA), a biopolyester with benign properties, such as biocompatibility and biodegradability. Since the cells may react to changing growth conditions and start to depolymerize PHA if the substrate becomes scarce, a quick analysis method is necessary to determine the optimal time for cell harvesting and polymer purification. In contrast, the GC analysis to quantify cellular PHA contents is laborious and lasts 2 to 3 days, which is far too long to be used for bioprocess control.

Recently, we described a robust method for the quantification of cellular PHA contents using flow cytometry: the cells are double-stained by BODIPY 493/503 and SYTO 62 to mark PHA and DNA, respectively.^[22] The advantage of this method is that PHA in cells can be clearly distinguished from hydrophobic debris (*e.g.* lysed cells), and most importantly, the time for at-line PHA analysis was less than 10 minutes (Fig. 6). Current research includes the automation of PHA quantification, thus providing online information on the cellular biosynthesis of PHA and helping minimize polymer loss by timely initiated actions.

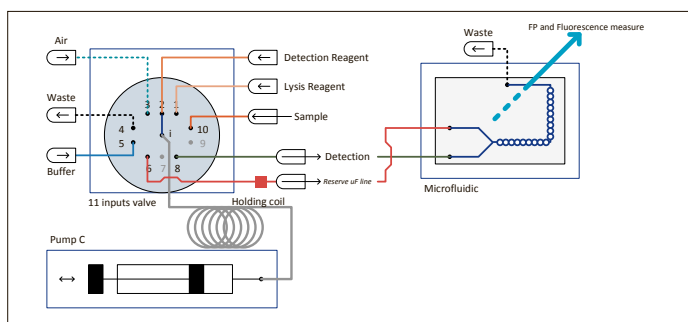


Fig. 5. A lab-on-valve setup coupled with microfluidics for at-line quantification of recombinant proteins using fluorescence polarization.

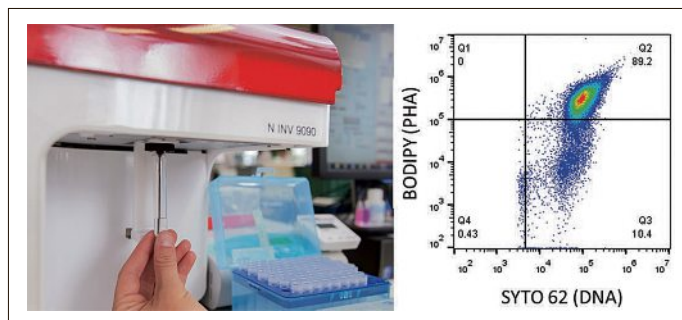


Fig. 6. At-line analysis of *Pseudomonas putida* KT2440 stained with fluorescent marker for PHA (BODIPY 493/503 and DNA (SYTO 62) using flow cytometry (left). 2D dot plot illustrating the heterogeneity of a *P. putida* KT2440 cell culture with respect to PHA content (Gate Q2 cells with high and Gate Q3 cells with low PHA content) (right).

Downstream processing. DSP unit operations such as precipitation, crystallization, or liquid–liquid extraction can be conducted in microfluidic systems. We recently isolated an IgG-1 monoclonal antibody from an industrial mammalian cell fermentation medium using an aqueous two-phase extraction system (ATPS) of following composition: 40% fermentation medium, 37% water, 8% sodium citrate, and 15% PEG 4000. Batch extraction trials at pH 6.0, allows 95% of the antibody to be collected with a purity of 59% in the bottom phase. Continuous extraction in a micromixing device (LTF-MS, 200 μ l volume, 1x1 mm channel) showed that extraction yields close to equilibrium values were obtained both at low (*e.g.* 10 g/h) and high (*e.g.* 450 g/h) flow rates, *i.e.* with long residence times or high turbulence, respectively. In between, partition equilibrium was not achieved, due to inefficient phase dispersion under laminar flow regime.

Received: June 1, 2017

- [1] T. Seifert, S. Sievers, C. Bramsiepe, G. Schembecker, *Chem. Eng. Process.* **2012**, *52*, 140.
- [2] T. L. LaPorte, C. Wang, *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 738.
- [3] a) N. Kockmann, P. Thenée, C. Fleischer-Trebes, G. Laudadioc, T. Noël, *React. Chem. Eng.* **2017**, ahead of print, DOI: 10.1039/C7RE00021A; b) N. Kockmann, *Chem. Ing. Tech.* **2012**, *84*, 715.
- [4] L. D. Proctor, A. J. Warr, *Org. Process Res. Dev.* **2002**, *6*, 884.
- [5] L. Grundemann, M. Schoenitz, S. Scholl, *Chem. Ing. Tech.* **2012**, *84*, 685.
- [6] N. Kockmann, M. Gottsponer, D. Roberge, *Chem. Eng. J.* **2011**, *167*, 718.
- [7] V. Hessel, S. Hardt, H. Löwe, 'Chemical Micro Process Engineering', Wiley-VCH, Weinheim, **2004**, 674 ff.
- [8] A. I. Stankiewicz, J. A. Moulijn, *Chem. Eng. Progr.* **2000**, *1*, 22.
- [9] https://www.heia-fr.ch/files/EN/EIA_en.pdf
- [10] <https://chemtech.heia-fr.ch/EN/presentation>
- [11] a) S. Abele, S. Höck, G. Schmidt, J.-A. Funel, R. Marti, *Org. Process Res. Dev.* **2012**, *16*, 1114; b) M. Mojzesova, M. Meciarova, R. Marti, R. Sebesta, *New J. Chem.* **2015**, *39*, 2573; c) E. Kilcher, S. Freymond, E. Vanoli, R. Marti, G. Schmidt, S. Abele, *Org. Process Res. Dev.* **2016**, *20*, 432.
- [12] K. N. Loponov, J. Lopes, M. Barlog, E. V. Astrova, A. V. Lapkov, A. A. Lapkin, *Org. Proc. Res. Dev.* **2014**, *18*, 1443.
- [13] D. H. Meikrantz, L. L. Macaluso, H. W. Sams, C. H. Schardin, A. G. Federici, U.S. Patent 5,762,800, **1998**.
- [14] A. Vienet, Bachelor Thesis HEIA-FR, Fribourg, **2015**.
- [15] P. Anastas, J. Warner, 'Green Chemistry: Theory and Practice', Oxford University Press, New York, **2000**, p. 30.
- [16] H. Jacobsen, in 'Handbook of Green Chemistry', Vol. 2, Eds.: T. Anastas, R. H. Crabtree, Wiley-VCH, Weinheim, **2013**, 93 ff.
- [17] C. Hutter, Ph.D. Thesis ETH Zürich No. 19188, **2010**.
- [18] S. Ueda, H. Nagasawa, *J. Am. Chem. Soc.* **2009**, *131*, 15080.
- [19] T. Leonhardt, A. Zogg, C. Hutter, J. Jeisy, W. Riedl, *Chem. Ing. Tech.* **2017**, *89*, 432.
- [20] A. Gössi, P. Schaer, W. Riedl, B. Schuur, 'Extraction and back-extraction of lactic acid from aqueous feeds using membrane supported reactive extraction', oral presentation at Aachener Membran Kolloquium, **2017**.
- [21] E. Condemi, D. Prim, R. Brönnimann, J. Fusco, S. Crelier, O. Mamula Steiner, J.-M. Segura, *CHIMIA* **2016**, *70*, 731.
- [22] S. Karmann, S. Follonier, M. Bassas-Galia, S. Panke, M. Zinn, *J. Microbiol. Methods* **2016**, *131*, 166.